In The Claims

Please amend the claims as follows:

1. (Currently Amended) A method of detecting microorganisms in a sample by means of detectable nucleic acid probe molecules comprising ~~the following steps~~:

a) fixing the microorganisms contained in the sample;

b) incubating the fixed microorganisms with ~~the~~ detectable nucleic acid probe molecules which are capable of hybridizing to a nucleic acid of the microorganism to be detected;

c) removing nonhybridized nucleic acid probe molecules;

d) separating hybridized nucleic acid probe molecules without using formamide under conditions that provide more detectable separated nucleic acid probe molecules than corresponding hybridized nucleic acid molecules separated using formamide, and

e) detecting the separated nucleic acid probe molecules, wherein the presence of the separated nucleic acid probe molecules correlates with the presence of the microorganism in the sample.

2. (Original) A method according to Claim 1, wherein the separated nucleic acid probe molecules in step e) are also quantified.

3. (Previously Presented) A method according to Claim 1, wherein the separation solution used in step d) is selected from the group consisting of water, buffered water, DMSO and SSC.

4. (Original) A method according to Claim 3, wherein the separation solution is 0.001 - 1.0 M Tris/HCl, pH 9.0 +/- 2.0.

5. (Previously Presented) A method according to Claim 3, wherein the separation solution is 0.01 M Tris/HCl, pH 9.0 +/- 2.0.

6. (Previously Presented) A method according to Claim 1, wherein step d) is carried out at a temperature of 50 to 100 °C.

7. (Previously Presented) A method according to Claim 1, wherein step d) is carried out at a temperature lower than 100 °C.

8. (Previously Presented) A method according to Claim 1, wherein step d) is carried out at a temperature of approximately 80 °C.

9. (Previously Presented) A method according to Claim 1, wherein the nucleic acid probe molecules are complementary to a chromosomal or episomal DNA, an mRNA or rRNA of a microorganism to be detected.

10. (Previously Presented) A method according to Claim 1, wherein the detectable nucleic acid probe molecules comprise nucleic acid probe molecules covalently bonded to a detectable marker.

11. (Original) A method according to Claim 10, wherein the detectable marker is selected from the group of the following markers:

a) fluorescence markers,
b) chemoluminescence markers,
c) radioactive markers,
d) enzymatically active group,
e) haptene,
f) nucleic acid detectable by hybridization.

12. (Previously Presented) A method according to Claim 1, wherein the microorganism is a single-cell microorganism.

13. (Previously Presented) A method according to Claim 1, wherein the microorganism is a yeast, a bacterium, an alga or a fungus.

14. (Original) A method according to Claim 13, wherein the microorganism belongs to the genus *Salmonella*.

15. (Previously Presented) A method according to Claim 1, wherein the sample is an environmental sample taken from water, soil or air.

16. (Previously Presented) A method according to Claim 1, wherein the sample is a food sample.

17. (Original) A method according to Claim 16, wherein the sample is taken from milk or milk products, drinking water, beverage, baked products or meat products.

18. (Previously Presented) A method according to Claim 1, wherein the sample is a medicinal sample.

19. (Original) A method according to Claim 18, wherein the sample is taken from tissue, secretions or fecal matter.

20. (Previously Presented) A method according to Claim 1, wherein the sample is taken from wastewater.

21. (Original) A method according to Claim 20, wherein the sample is taken from activated sludge, putrefactive sludge or anaerobic sludge.

22. (Previously Presented) A method according to Claim 1, wherein the sample is taken from a biofilm.

23. (Original) A method according to Claim 22, wherein the biofilm is taken from an industrial plant, is formed in purification of wastewater or is a naturally occurring biofilm.

24. (Previously Presented) A method according to Claim 1, wherein the sample is taken from a pharmaceutical or cosmetic product.

25. (Withdrawn) A kit for carrying out the method according to Claim 1, comprising:

a) at least one hybridization buffer,

b) at least one detectable nucleic acid probe for specific detection of a microorganism, and

c) at least one detectable nucleic acid probe for performing a negative control.

26. (Withdrawn) A kit according to Claim 25, comprising at least one specific probe for detection of bacteria of the genus Salmonella.

27. (Withdrawn) A kit according to Claim 26, comprising the nucleic acid probes

Salm63: 5'-TCGACTGACTTCAGCTCC-3'

and

NonSalm: 5'-GCTAACTACTTCTGGAGC-3'

or a nucleic acid probe that differs from Salm 63 and/or NonSalm by a deletion and/or an addition, whereby the ability of this probe to hybridize with Salmonella-specific nucleic acid is maintained, or a nucleic acid that can hybridize with the aforementioned nucleic acids.

28. (New) A method of detecting microorganisms in a sample by means of detectable nucleic acid probe molecules comprising:

a) incubating a sample comprising fixed microorganisms with detectable nucleic acid probe molecules which are capable of hybridizing to a nucleic acid of the microorganism to be detected;

b) removing nonhybridized nucleic acid probe molecules;

c) separating hybridized nucleic acid probe molecules without using formamide under conditions that provide more detectable nucleic acid probe molecules than corresponding hybridized detectable nucleic acid molecules separated using formamide; and

d) detecting the separated nucleic acid probe molecules, wherein the presence of the separated nucleic acid probe molecules correlates with the presence of the microorganism in the sample.

Remarks

Applicant has carefully reviewed and considered the Office Action mailed on June 3, 2003. Reconsideration and withdrawal of the rejections of the claims, in view of the amendments and remarks presented herein, is respectfully requested. Claim 1 is amended and claim 28 is added; as a result, claims 1-28 are pending in this application. The amendments are intended to advance the application and are not intended to concede to the correctness of the Examiner's position or to prejudice the prosecution of the claims prior to amendment, which claims are present in a continuation of the present application.

Claims 25-27 are withdrawn from further consideration as being drawn to a non-elected invention.

The amendments to claim 1 are supported in the specification at page 4, lines 9-12; page 5, lines 4-5; page 5, line 27 through page 6, line 4; page 7, line 34 through page 8, line 3; and page 8, line 27 through page 9, line 13.

New claim 28 is supported by originally-filed claim 1.

The 35 U.S.C. § 112, Second Paragraph, Rejection

The Examiner rejected claims 1-24 under 35 U.S.C. § 112, second paragraph, alleging that these claims are indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. The amendment to claim 1, to recite that the presence of separated nucleic acid probe molecules correlates with the presence of the microorganism in the sample, renders the Examiner's rejection moot. Thus, Applicant respectfully requests that the Examiner withdraw the 35 U.S.C. § 112, second paragraph, rejection.

The 35 U.S.C. § 102(b) Rejection

The Examiner rejected claims 1-2, 6, 9-13, 15-17, and 19-23 under 35 U.S.C. § 102(b) as being anticipated by Guillot et al. (WO 99/18234). This rejection is respectfully traversed.

Applicant respectfully submits that WO 99/18234 is not available as a reference under 35 U.S.C. § 102(b). For the sake of expediting prosecution of the instant application, if the Examiner were to present the rejection under 35 U.S.C. § 102(a), Applicant provides the

following remarks. Additionally, Applicant maintains the right to swear behind any reference which is cited in a rejection under 35 U.S.C. §§102(a), 102(e), and/or 103.

Anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration. *In re Dillon*, 919 F.2d 688, 16 U.S.P.Q.2d 1897, 1908 (Fed. Cir. 1990) (en banc), cert. denied, 500 U.S. 904 (1991). For anticipation, there must be no difference between the claimed invention and the reference disclosure, as viewed by a person of ordinary skill in the art. *Scripps Clinic & Res. Found. v. Genentech, Inc.*, 927 F.2d 1565, 18 U.S.P.Q.2d 101 (Fed. Cir. 1991).

Guillot et al. generally disclose a method in which microorganisms potentially present in a sample, e.g., a sample which has been fixed with an agent that maintains morphological integrity, are contacted with a RNA-targeted oligonucleotide probe, and the hybridized probes extracted, detected, and measured (page 4, lines 6-16 and page 6, lines 10-14). The extraction step is "performed by placing the microorganisms potentially present under conditions to denature enabling the denaturation of every probe specifically associated with its target sequence, notably in the presence of a probe-target denaturing agent such as one that will separate duplex DNA/DNA or DNA/RNA, and in particular the probe-target duplex under consideration, and at a temperature higher than the melting temperature of the probe under consideration, notably at a temperature of about 100°C" (page 8, lines 5-11). Guillot et al. continues by disclosing that the preferred denaturing agent is formamide and that extraction with formamide is performed at 100°C (page 8, lines 11-13). No other denaturing agents or protocols for detecting microorganisms in a sample are disclosed in Guillot et al.

Applicant respectfully asserts that while Guillot et al. generally disclose the use of denaturing agents to extract hybridized probes, there is nothing in the Guillet et al. reference that teaches or suggests the separation of hybridized nucleic acid probe molecules without using formamide under conditions that provide more detectable separated nucleic acid probe molecules than corresponding hybridized nucleic acid molecules separated using formamide.

Accordingly, the Examiner is respectfully requested to withdraw the rejection under 35 U.S.C. § 102.

The 35 U.S.C. § 103(a) Rejections

The Examiner rejected claims 1-13, 15-17 and 19-23 under 35 U.S.C. § 103(a) as being unpatentable over Guillot et al. in view of the Appendix of Roe et al. (Recombinant DNA Isolation, Cloning and Sequencing, 1996) and further in view of Kemp et al. (U.S. Patent No. 6,090,627). The Examiner also rejected claims 1-2, 6 and 9-24 under 35 U.S.C. § 103(a) as being unpatentable over Guillot et al. in view Sanders et al. (U.S. Patent No. 5,888,725). The Examiner further rejected claims 1-24 under 35 U.S.C. § 103(a) as being unpatentable over Guillot et al., in view of Roe et al., Kemp et al., and Sanders et al. These rejections are respectfully traversed.

The Examiner bears the initial burden of factually supporting any *prima facie* conclusion of obviousness. *In re Fine*, 837 F.2d 1071, 1074, 5 U.S.P.Q.2d 1596, 1598 (Fed. Cir. 1988). To establish a *prima facie* case of obviousness, three criteria must be met. First, the prior art reference (or references) must teach or suggest all of the claim limitations. Second, there must be some suggestion or motivation, either in the cited reference (or references), or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or combine reference teachings. Third, there must be a reasonable expectation of success. M.P.E.P. § 2142 (citing *In re Vaeck*, 947 F.2d 488. 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991)).

Guillot et al., as discussed above, do not disclose or suggest a method in which hybridized nucleic acid probes are separated without using formamide under conditions that provide more detectable separated nucleic acid probe molecules than corresponding hybridized nucleic acid molecules separated using formamide. Moreover, Guillot et al. teach that formamide is the preferred denaturing agent (see page 8, lines 11-13). Thus, Guillot et al. teach away from the present invention.

The portion of Roe et al. relied on by the Examiner in the Appendix of Roe et al. discloses a 10X denaturing buffer containing 200 mM Tris-HCl, pH 9.5, 1 mM EDTA, and 10 mM spermidine in double distilled water. From the title of Roe et al., "Protocols for Recombinant DNA Isolation, Cloning, and Sequencing," it appears that the solutions listed therein are for use in recombinant DNA isolation, cloning, and sequencing. The Appendix of Roe et al. does not disclose or suggest whether the solutions disclosed therein are suitable for use in a method of detecting nucleic acids of fixed microorganisms in a sample.

Kemp et al. disclose the sequence of the T-DNA of the octopine-type Ti plasmid found in *Agrobacterium tumefaciens* ATCC 15955, and the use of promoters and polyadenylation sites from pTi15955 to control expression of foreign structural genes (abstract). To sequence the T-DNA of pTi15955, Kemp et al. disclose that fragments of the T-DNA were subcloned into pBR322, and individual clones sequenced after the resulting plasmid DNA was digested, treated with calf intestinal phosphatase, denatured in 20 μM Tris-HCl, pH 9.5, 1 mM spermidine and 0.1 mM EDTA, and phosphorylated with kinase in the presence of radioactive ATP (column 28, lines 8-28). Kemp et al. do not teach or suggest a method of detecting the nucleic acid of fixed microorganisms in a sample.

Sanders et al. disclose "[a] method for detection, identification and/or quantification of target organisms of specific bacterial genus, species or serotypes, based on the occurrence of release of cell contents, particularly nucleotides, e.g., ATP, on lysis of bacterial cell walls on incubation with bacteriophages (phages) specific for them" (abstract). No other cellular contents are disclosed other than NAD, NADP, NADH, NADPH, ATP, ADP, cAMP, or cGMP (column 2, lines 32-45). Sanders et al. do not disclose or suggest a method of detecting microorganisms in a sample by hybridization of microbial nucleic acids with a nucleic acid probe.

With respect to the rejection of claims 1-13, 15-17 and 19-23 over Guillot et al., Roe et al. and Kemp et al., Roe et al. and Kemp et al. clearly do not remedy the deficiencies of Guillot et al. as none of the cited art teaches or suggests a method in which hybridized nucleic acid probes are separated without using formamide under conditions that provide more detectable separated nucleic acid probe molecules than corresponding hybridized nucleic acid molecules separated using formamide.

Moreover, the Appendix of Roe et al. discloses solutions for use in recombinant DNA isolation, cloning and sequencing protocols while Kemp et al. disclose a solution for use in denaturing DNA prior to end labeling that DNA. There is no evidence of record that the solutions disclosed in the Appendix of Roe et al. or Kemp et al. are suitable for use in Applicant's method. Thus, it is respectfully submitted that neither reference, alone or in combination with Guillot et al., provides one skilled in the art with a reasonable expectation that the instantly claimed methods of detecting microorganisms in a sample could have been successfully carried out.

Finally, the Examiner has not identified any suggestion or motivation to combine Guillot et al., Roe et al., and Kemp et al. in a manner necessary to arrive at the instant claims. Applicant respectfully submits that there is no motivation to combine the cited documents because the documents are from non-analogous art. Guillot et al. relate to detection of microorganisms in a sample while Roe et al. relate to recombinant DNA isolation, cloning, and sequencing, and Kemp et al. relate to sequencing and genetic engineering of pTil5955 DNA. Hence, Roe et al. and Kemp et al. involve nonanalogous art and should not be relied upon for a rejection under 35 U.S.C. § 103.

Thus, Applicant respectfully requests the Examiner to withdraw the rejection under 35 U.S.C. § 103(a) over Guillot et al. in view of Roe et al. and Kemp et al.

With respect to the rejection of claims 1-2, 6 and 9-24 over Guillot et al. in view of Sanders et al., Sanders et al. do not remedy the deficiencies of Guillot et al. as neither Guillot et al. or Sanders et al. teach a method in which hybridized nucleic acid probes are separated without using formamide under conditions that provide more detectable separated nucleic acid probe molecules than corresponding hybridized nucleic acid molecules separated using formamide. Accordingly, Applicant respectfully requests the Examiner withdraw the rejection under 35 U.S.C. § 103(a) over Guillot et al. in view Sanders et al.

To support the rejection of claims 1-24 over Guillot et al., Roe et al., Kemp et al., and Sanders et al., the Examiner alleges that "it would have been obvious to one skilled in the art at the time the invention was made to have practiced the claimed method taught by Guillot et al. (WO 99/18234, 15 April 1999), in view of Roe et al. (DNA isolation and sequencing, 1996), in further view of Kemp et al. (U.S. Patent 6,090,627) for the detection of microorganism in light of the method taught by Sanders et al. to specifically detect *Salmonella* in medicinal and pharmaceutical samples, for the expected benefit of a specific and rapid detection system for almost any bacteria in any environment" (page 10 of the Office Action).

Nevertheless, the methods disclosed in Guillot et al., Roe et al., Kemp et al., and Sanders et al. are quite different. Thus, one skilled in the art would not be motivated to combine the references in the manner suggested by the Examiner. Moreover, even if one skilled in the art did combine the references as suggested by the Examiner, the combination would not result in the instantly claimed method as none of the cited documents discloses or suggests a method in which

hybridized nucleic acid probes are separated without using formamide under conditions that provide more detectable separated nucleic acid probe molecules than corresponding hybridized nucleic acid molecules separated using formamide.

Furthermore, due to the differences in the disclosed methods, it is submitted that the cited documents would not have provided one skilled in the art with a reasonable expectation that the instantly claimed methods for detecting a microorganism in a sample could have been successfully carried out.

Therefore, Applicant respectfully requests the Examiner to withdraw the rejection under 35 U.S.C. § 103(a) over the combination of Guillot et al., Roe et al., Kemp et al., and Sanders et al.

Conclusion

Applicant respectfully submits that the claims are in condition for allowance and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's attorney ((612) 373-6959) to facilitate prosecution of this application.

If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 19-0743

Respectfully submitted,

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Date October 2, 2003

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CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: Commissioner of Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on this 2nd day of October, 2003.

Patricia A. Hultman _____

Name Signature